

DEFENSE HEALTH AGENCY
20.B Small Business Technology Transfer (STTR) Program
Proposal Submission Instructions

The Defense Health Agency (DHA) STTR Program seeks small businesses with strong research and development capabilities to pursue and commercialize medical technologies.

Broad Agency Announcement (BAA), topic, and general questions regarding the STTR Program should be addressed according to the DoD STTR Program BAA. For technical questions about a topic during the pre-release period, contact the Topic Author(s) listed for each topic in the BAA. To obtain answers to technical questions during the formal BAA period, visit <https://www.dodsbirsttr.mil/submissions/login>. Specific questions pertaining to the DHA STTR Program should be submitted to the DHA STTR Program Management Office (PMO) at:

E-mail - usarmy.detrick.medcom-usamrmc.mbx.dhpsbir@mail.mil
Phone - (301) 619-7296

PHASE I PROPOSAL SUBMISSION

Follow the instructions in the DoD Program BAA for program requirements and proposal submission instructions.

STTR Phase I Proposals have four Volumes: Proposal Cover Sheets, Technical Volume, Cost Volume and Company Commercialization Report. **Please note that the DHA STTR will not be accepting a Volume Five (Supporting Documents) as noted at the DoD BAA website.** The Technical Volume has a 20-page limit including: table of contents, references, letters of support, appendices, technical portions of subcontract documents (e.g., statements of work and resumes) and any other attachments. Do not include blank pages, duplicate the electronically generated Cover Sheets or put information normally associated with the Technical Volume in other sections of the proposal as these will count toward the 20-page limit.

Only the electronically generated Cover Sheets, Cost Volume and Company Commercialization Report (CCR) are excluded from the 20-page limit. The CCR is generated by the proposal submission website, based on information provided by small businesses through the Company Commercialization Report tool. Technical Volumes that exceed the 20-page limit will be reviewed only to the last word on the 20th page. Information beyond the 20th page will not be reviewed or considered in evaluating the offeror's proposal. To the extent that mandatory technical content is not contained in the first 20 pages of the proposal, the evaluator may deem the proposal as non-responsive and score it accordingly.

Companies submitting a Phase I proposal under this BAA must complete the Cost Volume using the on-line form, within a total cost not to exceed \$250,000 over a period of up to six months.

The DHA STTR Program will evaluate and select Phase I proposals using the evaluation criteria in Section 6.0 of the DoD STTR Program BAA. Due to limited funding, the DHA STTR Program reserves the right to limit awards under any topic and only proposals considered to be of superior quality will be funded.

Proposals not conforming to the terms of this BAA, and unsolicited proposals, will not be considered. Awards are subject to the availability of funding and successful completion of contract negotiations.

If a small business concern is selected for a STTR award they must negotiate a written agreement between the small business and their selected Research Institution that allocates intellectual property rights and rights to carry out follow-on research, development, or commercialization. Please refer to the DoD Instructions, section 4.2.f to view a “Model Agreement for the Allocation of Rights”.

RESEARCH INVOLVING HUMAN OR ANIMAL SUBJECTS

The DHA STTR Program discourages offerors from proposing to conduct human subject or animal research during Phase I due to the significant lead time required to prepare regulatory documentation and secure approval, which will significantly delay the performance of the Phase I award.

The offeror is expressly forbidden to use or subcontract for the use of laboratory animals in any manner without the express written approval of the US Army Medical Research and Development Command's (USAMRDC) Animal Care and Use Review Office (ACURO). Written authorization to begin research under the applicable protocol(s) proposed for this award will be issued in the form of an approval letter from the USAMRDC ACURO to the recipient. Furthermore, modifications to already approved protocols require approval by ACURO prior to implementation.

Research under this award involving the use of human subjects, to include the use of human anatomical substances or human data, shall not begin until the USAMRDC's Office of Research Protections (ORP) provides authorization that the research protocol may precede. Written approval to begin research protocol will be issued from the USAMRDC ORP, under separate notification to the recipient. Written approval from the USAMRDC ORP is also required for any sub-recipient that will use funds from this award to conduct research involving human subjects.

Research involving human subjects shall be conducted in accordance with the protocol submitted to and approved by the USAMRDC ORP. Non-compliance with any provision may result in withholding of funds and or termination of the award.

PHASE II PROPOSAL SUBMISSION

Phase II is the demonstration of the technology found feasible in Phase I. All DHA STTR Phase I awardees from this BAA will be allowed to submit a Phase II proposal for evaluation and possible selection. The details on the due date, content, and submission requirements of the Phase II proposal will be provided by the DHA STTR PMO. Submission instructions are typically sent toward the end of month five of the phase I contract. The awardees will receive a Phase II window notification via email with details on when, how and where to submit their Phase II proposal.

Small businesses submitting a Phase II Proposal must use the DoD SBIR electronic proposal submission system (<https://www.dodsbirsttr.mil/submissions/login>). This site contains step-by-step instructions for the preparation and submission of the Proposal Cover Sheets, the Company Commercialization Report, the Cost Volume, and how to upload the Technical Volume. For general inquiries or problems with proposal electronic submission, contact the DoD SBIR/STTR Help Desk (1-703-214-1333) or Help Desk email at DoDSBIRSupport@reisystems.com.

The DHA STTR Program will evaluate and select Phase II proposals using the evaluation criteria in Section 8.0 of the DoD STTR Program BAA. Due to limited funding, the DHA STTR Program reserves the right to limit awards under any topic and only proposals considered to be of superior quality will be funded.

Small businesses submitting a proposal are required to develop and submit a Commercialization Strategy (please refer to DoD Instructions, section 7.4) describing feasible approaches for transitioning and/or commercializing the developed technology in their Phase II proposal. This plan should be included in the Technical Volume.

The Cost Volume submitted must contain a budget for the entire 24-month Phase II period not to exceed the maximum dollar amount of \$1,100,000. These costs must be submitted using the Cost Volume format (accessible electronically on the DoD submission site), and may be presented side-by-side on a single Cost Volume Sheet.

DHA STTR Phase II Proposals have four Volumes: Proposal Cover Sheets, Technical Volume, Cost Volume and Company Commercialization Report. The Technical Volume has a 40-page limit including: table of contents, pages intentionally left blank, references, letters of support, appendices, technical portions of subcontract documents (e.g., statements of work and resumes) and any attachments. Do not include blank pages, duplicate the electronically generated Cover Sheets or put information normally associated with the Technical Volume in other sections of the proposal as these will count toward the 40-page limit.

Technical Volumes that exceed the 40-page limit will be reviewed only to the last word on the 40th page. Information beyond the 40th page will not be reviewed or considered in evaluating the offeror's proposal. To the extent that mandatory technical content is not contained in the first 40 pages of the proposal, the evaluator may deem the proposal as non-responsive and score it accordingly.

PHASE II ENHANCEMENTS

The DHA STTR Program has a Phase II Enhancement Program which provides matching STTR funds to expand an existing Phase II contract that attracts investment funds from a DoD Acquisition Program, a non-STTR government program or eligible private sector investments. Phase II Enhancements allow for an existing DHA STTR Phase II contract to be extended for up to one year per Phase II Enhancement application, and perform additional research and development. Phase II Enhancement matching funds will be provided on a dollar-for-dollar basis up to a maximum \$550,000 of STTR funds. All Phase II Enhancement awards are subject to acceptance, review, and selection of candidate projects, are subject to availability of funding, and successful negotiation and award of a Phase II Enhancement contract modification.

TECHNICAL AND BUSINESS ASSISTANCE (TABAs)

The DHA STTR Program does not participate in the Technical and Business Assistance (formally the Discretionary Technical Assistance Program). Contractors should not submit proposals that include Technical and Business Assistance.

The DHA STTR Program has a Technical Assistance Advocate (TAA) who provides technical and commercialization assistance to small businesses that have Phase I and Phase II projects.

PROTEST PROCEDURES

Please refer to the DoD Program Announcement for procedures to protest an Announcement. As further prescribed in FAR 33.106(b), FAR 52.233-3, Protests after Award should be submitted to:

Ms. Micaela Bowers
SBIR/STTR Contracting Officer
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DEFENSE HEALTH AGENCY STTR 20.B Topic Index

DHA20B-001	Refresher Training and Assessment in Austere Environments using a High Fidelity, Low Resource, Screen Based Virtual Patient Simulator
DHA20B-002	In-Ear Exposure Sensor with Integrated Noise Attenuation and Communications Capabilities
DHA20B-003	Develop and Demonstrate a Technology for Isolation of Bacteriophages with Enhanced Anti-Biofilm Activity

DHA20B-001 TITLE: Refresher Training and Assessment in Austere Environments using a High Fidelity, Low Resource, Screen Based Virtual Patient Simulator

RT&L FOCUS AREA(S): General Warfighting Requirements (GWR)

TECHNOLOGY AREA(S): Bio medical

OBJECTIVE: Design, develop, demonstrate, and evaluate the effectiveness of a mobile screen based virtual patient simulator with a high level of fidelity/realism allowing for just-in-time refresher training for emergency medical care for frontline service members in austere environments (land and sea).

DESCRIPTION: Service members, with a high degree of reliability, receive training in emergency medical care through established training courses: Advanced Trauma Life Support (ATLS), Advanced Care Life Support (ACLS), and Pediatric Advanced Life Support (PALS). Through these courses, service members receive the foundational training to provide frontline care in emergency medical situations in theater. The courses provide standardized training for what to do when faced with a decompensated patient. However, there is minimal training on how to recognize the early signs of decompensation in patients such as respiratory distress, shock and poor perfusion. As a result, there is a lack of demonstrated methods to measure readiness for these skills. Little to no implementation has occurred to enable corresponding assessments for use in refresher training. An additional shortcoming of the current training model is the lack of a practical, mobile and easy to use standardized refresher approach. As time passes from the initial training, knowledge and skills wane,^{1,2,3} decreasing the readiness of service members to appropriately respond when faced with medical emergencies.

The goal of this topic submission is to create a mobile screen based tool with established integrated readiness measurement and corresponding assessments for just-in-time refresher training around the early recognition and management of medical emergencies in adults and children with a variety of underlying causes. The system will have objective readiness measurement capability within a robust system of high resolution images, videos, simulation, augmented reality and virtual reality components to help learners recognize potential life threatening signs and symptoms in patients such as respiratory distress, shock, CNS injury, ocular trauma, poor skin perfusion, etc. These signs are not always as obvious as, for example, frank hemorrhage and lifesaving procedures may be delayed if early recognition is missed. The interest in addressing management of children comes from recent data demonstrating that approximately 11% of ICU bed days in theater were occupied by pediatric patients⁴. While the focus of service member medical training is and should be on caring for adults, the reality of current global conflicts and disasters is that the US military is the most adept medical facility providing care for all in distress. This effort will focus on both adults and children and can be used in military conflicts and humanitarian disasters.

In order to provide relevant and effective assessment for refresher training, the system must accurately display the presenting findings of Tactical Combat Casualty Care specific medical training scenarios such as blunt force or penetrating trauma (tension pneumothorax), airway compromise, shock (hemorrhagic or septic), and ACLS scenarios. The creation of realistic models that allow for 3D navigation on a mobile device will allow full assessment of clinical findings and enhanced fidelity of training. Additionally, the system must allow the service member to determine the problem at hand, identify, and apply the needed key interventions to facilitate transfer to a higher level of care or await advanced medical support. Solutions must address any limitations in simulation fidelity that in turn limit the ability to implement measures for readiness assessment.

In order to be effectively deployed in theater, the system needs to be accessible on readily available devices that are feasible for deployed service members (i.e. a screen based mobile phone or tablet). Additionally, to ensure reliable use in theater, the system needs to be accessible without com access,

facilitating use by those who truly are isolated from advanced support and would benefit the most from real time access to training.

PHASE I: Phase I will develop a proof of concept assessment capability for a simulation-based medical emergency recognition/response refresher tool. The medical case focus is how to recognize the early signs of decompensation in patients such as respiratory distress, shock and poor perfusion. Readiness measures should be developed and implemented within the training solution, and an initial concept design of the platform should be developed.

The following technological challenges should be addressed with proof-of-concept that demonstrate the feasibility of creating visually high fidelity representations of actual clinical presentations encompassing the key conditions faced by presenting service members and children in theater. Additionally, the feasibility of displaying necessary content on readily available screen based phones and tablets that allows for 3D evaluation of clinical findings should be addressed. Phase I solutions should also address the feasibility of integrating key content around recognition of findings and appropriate responses and the feasibility of delivering the content at the point of need, i.e. without the need for communication links in austere environments.

The intent of this phase is for the performer to produce the initial software, application design, and proof of concept that demonstrates the new innovation of the assessment platform that is being tested and indicate the types of risk anticipated. The performer will submit a final report and provide an initial demonstration describing the stage of the software development and application, along with details of what will be further developed in Phase II.

PHASE II: Building upon the development and lessons learned of Phase I, Phase II will focus on a proof of concept design for the robust refresher training platform in terms of functional requirement, content design, architecture design, component design, coding, testing of the platform, and delivery of the platform.

Currently available government-approved screen based phones and tablets that are utilized in theater should be identified as candidate devices for testing the developed platform. It will additionally need to demonstrate medical accuracy and user functionality for all necessary training scenarios included within the Tactical Combat Casualty Care specific medical training scenarios as well as common pediatric presenting conditions.

The Phase II product will need to demonstrate the usefulness of the platform developed with appropriate collection of usability and reliability data from participants who would use the product in the demonstration phase. The Phase II product will need to demonstrate readiness measurement solutions that have sufficient discrimination. Solutions should provide data to enable assessment and modeling of initial acquisition, maximum proficiency, retention, and relearning. The performer will provide a demonstration of the product and discuss potential Phase III developments.

PHASE III: Concluding in Phase III, the performer will have built a viable, commercially available software product accessible in a downloadable application that can be used to train and assess in any location that medical first responders are deployed. Preferably, the capability will be based on state of the art software and hardware principles, use validated data from publicly available sources, and be clinically accurate and relevant to allow for effective refresher training. Solutions should conform to allowable (deployable) technologies (i.e., not Bluetooth reliant) and should function in austere environments.

The system will perform without degradation due to dust, sand, rain, humidity, wind, extremes in temperature and electromagnetic interference and will withstand repeated drops on all axes.

Environmental performance specifications will be determined before final design. A successful system will be expected to pass MIL STD 810G certification on all approved system specifications. The training solution is not anticipated to be permanently installed inside an aircraft; therefore standards from the Joint Enroute Care Equipment Test Standard must be tailored from the original guidelines.

It is anticipated that DoD customers will include Medical Department personnel, TCCC participants, Reserve components, and Federal Agencies involved in disaster assistance.

Commercial markets that could benefit from this novel product would include: emergency/first responder training, undergraduate/graduate medical training, and nursing training. Societies responsible for first responder training such as the AHA and institutions responsible for local emergency medical services support and training could benefit from such a product.

Upon completion, the performer will submit a final report describing the software application and the demonstration results.

REFERENCES:

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2. Ciurzynski, Susan M. PhD, RN-BC, MS, PNP et al. (2017). Impact of Training Frequency on Nurses' Pediatric Resuscitation Skills. *Journal for Nurses in Professional Development*. 33(5):E1-E7, September/October 2017.
3. Smith K. K., Gilcreast D., Pierce K. (2008). Evaluation of staff's retention of ACLS and BLS skills. *Resuscitation*, 78, 59-65. doi:10.1016/j.resuscitation.2008.02.007
4. Borgman M, Matos RI, Blackburne LH, Spinella PC. Ten years of military pediatric care in Afghanistan and Iraq. *J Trauma Acute Care Surg*. 2012 Dec;73(6 Suppl 5):S509-13. doi: 10.1097/TA.0b013e318275477c.

KEYWORDS: Refresher Training; Recognition and Management; Readiness Assessment; Virtual Patients; Decompression in Patients

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DHA20B-002 TITLE: In-Ear Exposure Sensor with Integrated Noise Attenuation and Communications Capabilities

RT&L FOCUS AREA(S): General Warfighting Requirements (GWR)

TECHNOLOGY AREA(S): Bio medical

OBJECTIVE: Develop, demonstrate, and deliver an inner-ear sensor device that integrates three functions: 1) sensing and recording head response to both blast and blunt impact events, 2) communications, and 3) continuous and impulse noise attenuation. This STTR aims to develop a device that provides the ability to monitor multiple types of exposure (blunt impact and blast) to Service Members during training and combat operations for potential traumatic brain injuries.

DESCRIPTION: The Defense and Veterans Brain Injury Center (DVBIC), in conjunction with the Armed Forces Health Surveillance Center, tracks traumatic brain injury (TBI) diagnoses for all U.S. military personnel (deployed and non-deployed). There were 383,947 TBI diagnoses of all severities between 2000 and Q1-2018 (DVBIC, 2019). Depending on the severity of the TBI, symptoms may last from a couple of days to multiple years following the injurious event. Moreover, repeated TBIs may result in more severe and long-term consequences. There have been several attempts to record the exposure conditions related to mild traumatic brain injuries (mTBI) in the military, beginning with an effort directed by the Vice Chief of Staff of the Army (VCSA) in 2007. Existing technologies that are usable in a military environment only measure blast exposure or suffer from poor coupling to the head and require substantial post-processing of the data to correlate the sensor motion to head motion. Commercially available technologies developed for athletics environments are not broadly compatible with the military environment due to interactions with protective equipment and coupling to the head under extreme conditions (Rooks et al., 2015). Additionally, no current capability (military or civilian) integrates the ability to measure both impact and blast exposures in a single package to an acceptable degree.

Additionally, currently available commercial technologies suffer from insufficient battery life and a large overhead of personnel to manage the use of the technology (Rooks et al., 2015). Studies have shown that sensors deeply inserted into the ear canal can have better coupling to the head and can represent head motion accurately (Salar, 2008; Panzer et al., 2009; Christopher et al., 2013). Additionally, in-ear sensors have been used in the motorsports industry to monitor driver head accelerations during crash events (Knox et al., 2008) as well as measuring head accelerations in rough stock riders (Mathers et al., 2012). Current in-ear sensor systems do not combine the sensor technology with other essential functions that an earpiece must provide to the military Service Member: communications and continuous steady-state and impulse noise attenuation.

This STTR aims to develop a device that provides the ability to monitor Service Members during combat operations for potential traumatic brain injuries. Additionally, the device will integrate with existing Service Members' communication systems, while not hindering communication and providing noise attenuation. This increased monitoring will allow Soldiers to receive medical attention sooner to address potential injuries prior to any additional or compounding injuries. By quickly addressing injuries, Soldier return-to-duty may be accelerated, thus maintaining combat power and increasing Soldier lethality.

PHASE I: Develop device concepts and designs that integrate the desired functions of recording blast and blunt impact exposure, providing communications ability, and providing hearing protection. Additionally, perform a technical trade assessment of the conceptual designs, to include: sensor recording requirements, communications requirements, and noise attenuation requirements for military-specific applications.

The proposed device must integrate all three functions (ability to sense and record head response to blast and blunt exposures, communication, and continuous steady-state and impulse noise attenuation) into a

single device. Ideally, the proposed device should be electronically readable, scan-able, or transmittable to DOD approved devices and to manage data and alert team members and medics of a potentially injurious exposure. The device should be capable of integrating with currently fielded Department of Defense (DoD) communications systems, to include drawing power from the radio battery packs (if required for operation) and transmitting data through secure communication channels. If self-powered, the device must have a battery life of at least 72 hours of continuous operation with the ability to be recharged. The device should have minimal power consumption, be low-weight, capable of accommodating the range of Soldiers' ear sizes, comfortable for extended wear, durable, cheap, and reusable. The target storage capability for the device is 1000 time-trace events before downloading. The target response range for the device is ± 500 G, $\pm 6,000$ deg/sec, and ± 100 psi. The target sampling rate for the device is 100,000 Hz, with a minimum duration of 100 ms. Data acquisition should have a minimum of 16-bit resolution, and measurement error should be less than 0.01% for all sensors. Trigger threshold should be adjustable in sensor configuration settings. The timestamp should be accurate to less than 1-second and should have no more than 1-second of drift for a minimum of seven days without synchronizing with a source. Sensor settings (i.e., unique identifier, timestamp, trigger threshold) and data should be stored on non-volatile memory. A time-stamp indicator should be documented before device power depletion and for every recorded event.

Work in Phase I should demonstrate the field compatibility of the design by delivering two weight and geometrically representative mock-ups. Additionally, work completed in Phase I should demonstrate the ability to integrate all three desired functions into a single platform. Along with the mock-ups, the contractor shall deliver documentation on the most promising concept design(s), anticipated developmental testing requirements, proposed test procedures, and preliminary data to demonstrate functionality, compatibility, working principles, and use. The contractor will develop the work plan for subsequent development and prototyping.

PHASE II: Using results from Phase I, construct and demonstrate the operation of a prototype that integrates the three desired functions of recording exposure, communications, and hearing protection. The prototype will also include any hardware/software interfaces that are required for system functionality (data download and processing). Upon successful demonstration of an operational prototype to government representatives, mature the selected design for wear by Service Members and construct working prototypes for limited field testing and evaluations.

Required Phase II deliverables include: 12 working prototype units and associated hardware/software interfaces required for system functionality, documentation on use of the device, a report on the limited field testing and evaluations, and a final report on device design and validation testing.

PHASE III: Mild TBI and the ability to identify potentially injurious events is not solely a military concern. Significant efforts are being made to improve the ability to identify and diagnose mTBI both within the military and commercially. While sensors have been developed and used extensively in athletics, there remains a gap on the market for wearable sensor technologies that can be used occupationally, whether that is in the military (operationally) or commercially. For instance, law enforcement personnel are routinely exposed to similar threats as many military applications, and there is no current sensor that can measure both blast and blunt impact sufficiently within either group. Commercially, an inner-ear multipurpose sensor could become standard issue equipment for the civilian law enforcement community, bomb squads, miners, flight-line personnel, and construction personnel, to name a few occupations. For use with many of the civilian and law enforcements occupations, additional development may be required. Many of the non-military environments use wireless data transmission (e.g., Bluetooth, local wifi networks, etc.) and may use commercially available radio and communication systems rather than DOD approved systems. During phase III, the performer will be encouraged to further develop the prototype device to facilitate use with non-DOD occupations and communities.

Ongoing research efforts in the DOD are using COTS devices that partially fill the gap; however, the devices available are not final solutions for occupational monitoring in training and operational environments. A successfully developed sensor would be integrated into ongoing research, surveillance, and evaluation efforts conducted through the Environmental Sensors in Training (ESiT) program as well as efforts under development to address the 2018 and 2020 National Defense Authorization Acts calling for occupational monitoring of blast exposure.

REFERENCES:

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KEYWORDS: Concussion, Blast, Impact, Head impact, mTBI, TBI, Environmental Sensor, Noise Attenuation, Earphones, Headset, Radio Headset

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DHA20B-003 TITLE: Develop and Demonstrate a Technology for Isolation of Bacteriophages with Enhanced Anti-Biofilm Activity

RT&L FOCUS AREA(S): General Warfighting Requirements (GWR)

TECHNOLOGY AREA(S): Bio medical

OBJECTIVE: Develop and demonstrate a technology to enable rapid enrichment and isolation of bacteriophages (phages) with enhanced biofilm dispersal activity for the treatment of recalcitrant infections of the Warfighter in the post-antibiotic era.

DESCRIPTION: Multidrug-resistant organisms (MDRO) have spread worldwide and triggered a major public health crisis. U.S. military service members wounded in combat are susceptible to infection by MDRO, including biofilm-mediated infection, at a much higher rate than civilian population due to penetrating combat wounds being accompanied by foreign body inoculum (metal fragments, rocks, dirt), large zones of bone and soft tissue disruption, nerve damage and localized ischemia (tourniquet /edema). Biofilms may begin to form in wounds in as little as a few hours post-infection, and their extracellular matrices provide the bacteria protection from the human host immune response and antibiotic therapy. Furthermore, the current concepts of war moving towards urban dense terrain (UDT) and multi-domain operations (MDO) are expected to generate complex wounds that will require advanced prolonged field care and stabilization when tactical evacuations to robust rear element medical care infrastructures are delayed. Such a delay in evacuation and limit on comprehensive care for combat wound orchestrate the ideal conditions for biofilm formation in severely traumatized tissue. To make matters worse, the potential for life threatening infection by MDRO, particularly biofilm-mediated infection, is even higher under the MDO and UDT settings and the need for novel solutions is urgent. ESKAPEE pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Enterobacter* spp., and *Escherichia coli*) frequently colonize healthy military personnel (1) and are causative agents of persistent infections of traumatic and burn wounds that are prone to biofilm formation and multidrug resistance (2). A scarcity in effective antibiotic therapy options warrants the development of alternative potent antibacterials, e.g. phages.

Phages are natural viruses that specifically kill bacteria resistant to antibiotic treatment and have been shown to be able to disperse biofilms by their polysaccharide depolymerase activity and to efficiently kill bacteria in biofilms both in vitro and in vivo (3). Phages exhibit extraordinary specificity to target bacterial strains and can eliminate them without affecting normal microflora. In vitro studies have shown that bacteriophage can penetrate mature biofilms and cause bacterial cell lysis. A major advantage of phage therapy is the ability to exploit the constant natural evolution of phages to overcome phage resistance, infect and kill the host bacteria. Phages have demonstrated high efficacy against ESKAPEE infections in laboratory, domestic and farm animals and promising data in expanded access treatment of humans and even in recent clinical trials, especially in combination with antibiotics (4,5). Phages are becoming a very important adjunct therapy against MDR bacterial infections in civilian and military patients.

The gold standard method for isolating phages is via planktonic growth of bacteria in the presence of a phage source (e.g., sewage). The phages isolated under such conditions would bind to and infect bacteria displaying receptors expressed during planktonic growth, which will not necessarily be the same receptors expressed during biofilm growth. Thus, taking these planktonic growth-isolated phages, assembling them in cocktails and attempting to treat biofilm-mediated infections could be a flawed methodology.

The purpose of this STTR is to enable relatively rapid phage enrichment, screening, and isolation on biofilm of a permissive strain of interest. The end products of the system sought through this process are phages with enhanced biofilm degradation activity against strains of interest. This capability will

drastically improve force health protection at large and will more directly enable the formulation of better therapeutic phage cocktails using diverse phages with broad killing spectra isolated from bacterial biofilms, to target biofilm-mediated infections.

Users should have the freedom to select permissive and target screening strains of interest. The technology may be, but is not limited to, micro-filtration systems, microfluidics, centrifugation, nano-materials, gel or polymer matrix or any combination of relevant and novel technologies. The device can be a closed or open modular system. The following features will be critical to consider when proposing a technology:

- 1) System should enable users to select and input permissive strains of choice for optimized biofilm formation, and to propagate phages on target strains of choice for activity assessment
- 2) System should perform the enrichment on bacterial biofilms and isolate viable phages with enhanced polysaccharide depolymerase and biofilm dispersal activity
- 3) System should enable isolation of phages against multiple target strains of interest simultaneously
- 4) System should enable quantitative screening of phage activity against biofilms of multiple target strains of interest simultaneously
- 5) Portability of system is preferred but not a requirement
- 6) Reusable design of consumables are preferred features but not a requirement

PHASE I: This phase should focus on the design of a proof-of-concept prototype technology/device that enables phage enrichment (propagation) on biofilms to produce viable phage particles with enhanced anti-biofilm activity. During this phase, STTR performer should focus on maturing stable biofilm formation on at least, but not limited to, two strains (i.e. permissive strain) of choice on design of choice for screening phages. Phages can be isolated from sewage, environmental waters such streams and ponds, farm run-offs, and harbors. Anticipated components of new device may include, but not limited to, 1) a consumable that allows biofilm growth of permissive cell; 2) a sensor for a qualitative or quantitative assessment of anti-biofilm activity of phages compared to control; 3) a smart device to analyze and interpret data; 4) a method to recover and preserve phages for further testing and validation. System does not need be integrated at this stage but should have a workflow. However, at the end of this phase, working prototype(s) should demonstrate permissive strain input access, mature biofilm formation, and phage propagation capability of the system. Performance (i.e. turnaround time to enriched phages) should be compared to classical manual in vitro approaches over 24, 48, and 72 hrs. Ideally, with regards to portability, performer should also explain how the proposed device can be made suitable for use in a field environment with further development (i.e. the field- testable system should not exceed 30 lbs, self-contained and none of its dimensions should exceed 16 inches, with minimal battery operation for 12 hrs.) The size and cost of the consumable components should be no greater than the currently available fluidic biochips on the market. Provide a written plan for Phase II to reduce the size, simulate field use, and cost of the consumable component. The goal is to reduce size of consumable to less than \$10 per test if performer is unable to design reusable consumables.

PHASE II: During this phase, the technology/device should be integrated into a system. The workflow from Phase I should be refined to expand on the proof-of-concept into a product that enables high-throughput screening of phages against biofilms of diverse MDRO strains of choice. STTR performer should address features listed as critical features of technology include quantitative assessment of anti-biofilm phage activity, portability of system and reusability of consumables. This testing should be controlled, rigorous, and reproducible. Here, STTR performer may choose, but not required, to coordinate with WRAIR subject matter experts to freely collaborate in optimizing and validating system. This phase should also demonstrate evidence of commercial viability of the product.

PHASE III: This phase should focus on scaling production, marketing of technology to distributors, and contracts. Accompanying application instructions, simplified procedures, and training materials should be drafted in a multimedia format for use and integration of the product into market. The end-state for this product is a commercially viable technology that will be incorporated to the preventive medicine and medical surveillance mission for Force Protection by the Department of Defense by establishing a National Stock Number (NSN) as the first step towards the potential inclusion into appropriate "Sets, Kits and Outfits" that are used by deployed medical forces in the Defense Acquisition System. Furthermore, performer should pursue a commercial path to democratize phage-harvest efforts across medical institutes, bio-pharma and educational institutes.

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